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INHIBITION OF PACLITAXEL AGAINST NEUROGLIOMA CELLS U251 GROWTH AND ITS MECHANISM

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Abstract

Background: Glioma is the most common primary tumor of the central nervous system, and accounted for about 70% of primary tumors.
Materials and Methods: In the study, antitumour activity and mechanism of paclitaxel was investigated. Different concentrations of paclitaxel (200, 300, 400 μmol/L) was treated in neuroglioma cellsU251.
Results: Paclitaxel significantly inhibited neuroglioma cells growth, and promoted its apoptosis. Paclitaxel can block tumour cells in the G2/M phase. In addition, apoptosis-related genes caspase-3 and bax expressions were increased after paclitaxel treatment.
Conclusion: Our work indicated that paclitaxel displayed strong anti-tumour activity.

Keywords: paclitaxel, antitumour, apoptosis, cell cycle

Introduction

Glioma is the most common primary tumor of the central nervous system, and accounted for about 70% of primary tumors. Malignancy degree of glioblastoma is highest, and accounted for 50% of all gliomas. At present, main method of the treatment is surgery, and also have some auxiliary ways, e.g. chemoradiotherapy and radio-chemotherapy. But they are not good effect. Postoperative recurrence and death rate are higher (Okunaga et al, 2006).

Paclitaxel is a high efficiency, low toxicity, broad spectrum natural plant anticancer medicine. A large number of studies show that paclitaxel can inhibit the proliferation of a variety of tumor (Xu et al, 2013). In this study, we evaluated inhibitory effect of paclitaxel and cisplatin on neuroglioma growth.

Material and methods

Inhibition rate analysis of tumour cells

Cell viability was evaluated using the MTT 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl-2-(4-sulfo-phenyl)-2H-tetrazolium) (Sigma–Aldrich, France) assay. The different concentration of Paclitaxel (200, 300, 400 μmol/L) were plated in a total volume of 200 mL in 96-well plates (Becton Dickinson, France). The wells containing only tumour cells U251 were used as control groups. Following48 h of incubation at 37 ^{o}C, 0.02 mL MTT was added to each well and the plates were incubated for 2 h, andthen 100 mL of DMSO was added to dissolve the blue formazan crystals. The absorbance was measured by spectrophotometry at545 nm.

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Apoptosis rate and cell cycle distribution

Annexin V - FITC/PI double parameter method was used. Light sources were 488 nm argon ion laser. FITC emits the green fluorescence after excitation, and PI emits red fluorescence. 1×10^4 cells were collected from each specimen. Data are analyzed by relevant software.

Tumor cells ($2 * 10^5$) were cultured with vehicle, Paclitaxel (200, 300, 400 μmol/L) at 37 ^oC for 48 h. Cells were collected, washed with PBS and suspended, then fixed with 99.5% ethanol at 4 ^oC for 2 h. Then, cells were treated with RNase at 37 ^oC for 1h, and reacted with PI (propidium iodide, PI, 500 mg/ml, Sigma Chemical Co.) at room temperature for 10 min. DNA changes of cell cycle were measured by flow cytometry.

Western blot analysis

Tumor cells ($2 * 10^5$) were cultured with vehicle, different concentrations of Paclitaxel (200, 300, 400 μmol/L) at 37 ^oC for 48 h. Cells were collected to extract proteins. The 20 μg protein sample was added into 7.5%SDS-PAGE gel for electrophoresis and the separation of proteins were then transferred to the PVDF film. The membranes were blocked with 5% skim milk for 1 h and incubated overnight with the following specific antibodies: β-actin (1:1000), caspase-3 (1:1000), bax (1:1000), bcl-2 (1:1000) overnight at 4 °C. The membranes were then incubated with the appropriate secondary antibody and were washed again with TTBS at room temperature thrice (10 min each wash). Finally, the blots were detected with chemiluminescence color display system (Amersham Life Science, Tokyo, Japan) .

Data analysis

Data was presented as mean ± S.D. Experiment results are analyzed using SPSS17.0 for Windows statistical software. Single factor analysis of variance was performed using ANOVA. The p-values ≤ 0.05 were considered as statistically significant.

Results and Discussion

The inhibitory effects of Paclitaxel on breast cancer cells, neuroglioma cells and esophageal cancer cells are significant. Moreover, berberine doesn't cause side-effect (Simpson & Plosker, 2004). The antitumour mechanism of Paclitaxel include to Inhibit the growth of tumor cells and promote their apoptosis; affect cancer gene and tumor suppressor gene; Induce malignant tumor cells to differentiate into normal cells; Inhibit tumor angiogenesis and tumor cell metastasis. As shown in Fig 1, inhibition rate (%) of Paclitaxel against tumour cells increased with increasing concentration of berberine. At high concentration, inhibition rate (%) of Paclitaxel against tumour cells was 46.03±2.95%.

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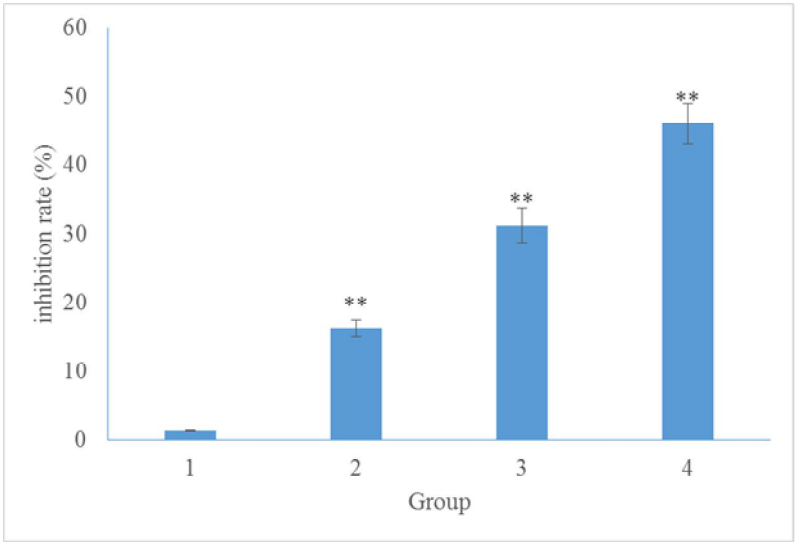

Figure 1: Paclitaxel inhibiting tumour cell growth

1. 0 µmol/L, 2. 200 µmol/L, 3. 300 µmol/L, 4. 400 µmol/Lµmol/L; ** P <0.01, compared with group 1.

As shown in Fig 2, apoptosis rate (%) of tumour cells increased with increasing concentration of Paclitaxel. At high concentration, apoptosis rate (%) of tumour cells was 44.27±3.17%.

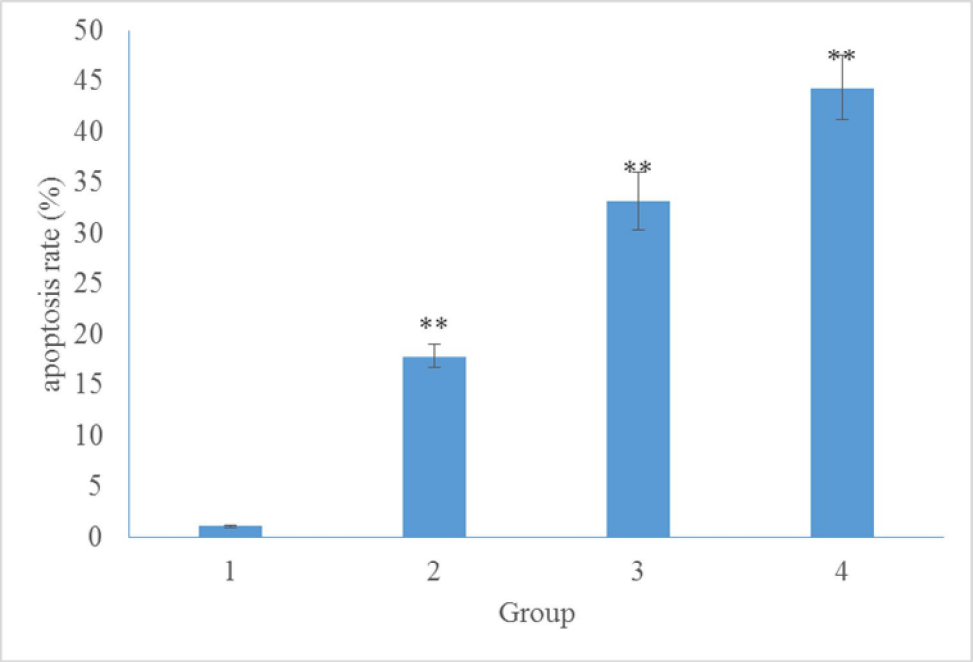

Figure 2: Paclitaxel promoting tumour cell apoptosis

1. 0 µmol/L, 2. 200 µmol/L, 3. 300 µmol/L, 4. 400 µmol/Lµmol/L; ** P <0.01, compared with group 1.

Compared with untreated control group, berberine treatment significantly decreased G0/G1 phase, and increased G2/M phase in tumour cells. The effect shows a dose-dependent manner. However, Paclitaxel treatment didn't significantly affect S phase in tumour cells. Its action mechanism might be to promote tumour cells apoptosis by increasing proliferation cycle G2/M phase, and decreasing G0/G1 phase.

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Table 1: Paclitaxel affecting tumour cell cycle distribution

Dose (μmol/L)	G0/G1 (%)	G2/M (%)	S (%)
0	45.2±2.88	8.59±0.38	46.21±2.47
200	39.8±1.95 *	15.39±1.13 **	44.81±2.81
300	28.17±1.84 **	23.81±1.21 **	48.02±3.06
400	23.47±1.72 **	30.28±1.44 **	46.25±3.28

*P<0.05, ** P <0.01, compared with group 1.

Caspase 3 is the key effector molecule in the apoptosis induced by multiple stimuli. Under normal conditions, caspase-3 in cytoplasm has no activity. When the cell apoptosis, zymogen will be activated into caspase-3. Activated caspase-3 may inactivate proteins and kinases associated with cell structure, cell cycle, and DNA repair so that the cancer cell proliferation rate slows down or induces the cell to go to death (Maiyo et al, 2016). Antiapoptosis mechanism of bcl-2 is achieved by changing the redox state and membrane potential of mitochondria, regulating the permeability of mitochondrial membrane, binding of pro-apoptotic protein Apaf-1 to inhibit the activity of ASP. At last, it protects cells from apoptosis. BAX is a water-soluble protein associated with BCL-2, a gene that promotes apoptosis in the BCL-2 gene family. Excessive expression of BAX can protect the cell from the protective effect of BCL-2 and make the cell die (Ghosh et al, 2012). As shown in Fig 3, compared with untreated control group, Paclitaxel treatment significantly increased pro-apoptotic proteins caspase-3 and bax, and decreased anti-apoptotic proteins bcl-2 in a dose-dependent manner. Our results indicate that Paclitaxel promotes tumour cell apoptosis by regulating caspase-3, bax and bcl-2 proteins expression.

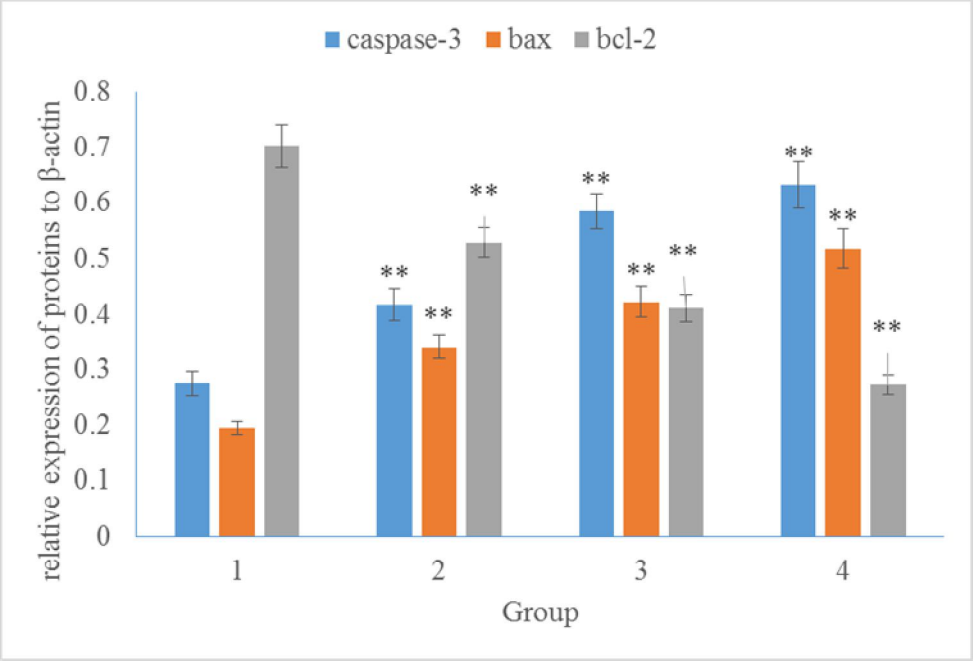

Figure 3: Paclitaxel promoting caspase-3, bax proteins expression and inhibiting bcl-2 protein expression in tumour cells 0 μmol/L, 2. 200 μmol/L, 3. 300 μmol/L, 4. 400 μmol/Lμmol/L; ** P <0.01, compared with group 1.

Conclusion

It appears that paclitaxel plays its strong antitumour effect against neuroglioma cellsU251 growth. These study

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strengthen its traditional use in brain tumour disease.

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